Original Article

Lung functions and oxidative status in sickle cell disease and sickle cell trait

Nitin Ashok John, John Jyoti[1], Dhanapal Niraimathi, Sajja Venkatappa Umadevi, Krishnan Puviarasan, Madhura Abhishek[2]

Department of Physiology, Indira Gandhi Medical College and Research Institute, Kadhirkhamam, [1]Department of Biochemistry, Pondicherry Institute of Medical Sciences, Puducherry, [3]Department of Pediatrics, Prestige Hospital, Nagpur, Maharashtra, India

Abstract

Background and Aim: Sickle cell disease (SCD) is a genetic hemoglobinopathy. SCD is associated with increased production of reactive oxygen species resulting in hemolysis, endothelial dysfunction, cell adhesion, and vaso-occlusion. Pulmonary disease is a major cause of morbidity and mortality in SCD. Hence, in the present study we have planned to assess the pro-oxidant and anti-oxidant status and lung function tests in patients with SCD, and sickle cell trait (SCT).

Methods: Freshly diagnosed cases of SCD and SCT (based on sickle cell hemoglobin [HbSS] and normal hemoglobin [HbA] pattern by hemoglobin electrophoresis) were included in the study. The lung function tests and oxidative stress parameters namely plasma malondialdehyde (MDA), whole blood superoxide dismutase (SOD) and plasma Vitamin C levels were assessed.

Results: Forced vital capacity, forced expiratory volume in 1 s, maximum mid-expiratory flow rate, peak expiratory flow rate and maximum voluntary ventilation were reduced in patients with SCD and SCT. MDA and SOD levels increased significantly and Vitamin C levels decreased significantly in both homozygous and heterozygous cases of sickle cell anemia.

Conclusion: The lung functions were compromised, and oxidative stress was increased in patients with SCD and SCT. The changes were more in SCD.

Key words: Lung function, oxidative stress, sickle cell disease, sickle cell trait

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INTRODUCTION

Hemoglobinopathies are inherited abnormalities of hemoglobin synthesis, characterized by structurally abnormal hemoglobin. Sickle cell disease (SCD) results from a single mutation in the β-globin chain due to the substitution of valine for glutamic acid at the sixth amino acid position. As a result of mutation, there is production of abnormal hemoglobin S (HbS). Apart from the homozygous SCD (HbSS), other forms such as abnormal hemoglobin C and HbS with β-thalassemia also exist.[1,2] The pathogenesis of SCD is mainly due to the polymerization of deoxygenated HbS which alters the normal biconcave disc shape into a rigid unstable cell leading to intravascular hemolysis and release of hemoglobin into the plasma. As there is repeated polymerization in SCD it can lead to a cyclic cascade causing blood cell adhesion, vaso-occlusion, and ischemia-reperfusion injury. These manifestations alter the levels of reactive oxygen species (ROS) and antioxidants.

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Sickle cell anemia (SCA) is associated with increased oxidative stress. Previous reports have shown sickle cell erythrocytes produce twice as much as superoxides, hydrogen peroxides and hydroxyl radicals as compared to normal healthy controls.[3,4] SCD is associated with inflammatory responses in many organs, and also produces secondary diseases such as acute chest syndrome (ACS), pulmonary hypertension (PHT) and stroke. There are reports showing that SCD patients have frequent episodes of chest infections and decreased lung functions.[5-8]

The incidence of SCA being very high in Vidarbha region

Address for correspondence: Dr. Nitin Ashok John, Department of Physiology, Indira Gandhi Medical College and Research Institute, Kadhirkhamam, Puducherry - 605 009, India. E-mail: drnitinjohn@yahoo.co.in

of Maharashtra and only very few studies have been carried out till date to assess the pulmonary functions and oxidative stress in patients with SCD and sickle cell trait (SCT). Also, there are no studies correlating the lung functions with oxidative status in patients with SCD and SCT in Vidarbha region of Maharashtra. Therefore, in the present study we have planned to assess the pulmonary function status, pro-oxidant and anti-oxidant levels in SCD and SCT patients between the age group of 15-45 years.

MATERIALS AND METHODS

The present study was carried out in the Department of Physiology and Biochemistry, Indira Gandhi Medical College, Nagpur. SCD and SCT patients were recruited from the regional haemoglobinopathy detection center and medicine OPD of Indira Gandhi Medical College and Mayo Hospital, Nagpur. The project was approved by the Institute ethics committee. The study included 45 healthy controls, 45 cases of SCT and 30 cases of SCD between the age group of 15-45 years. SCD and SCT patients were randomly selected from sickle cell unit after confirmation of HbSS and normal hemoglobin (HbA) pattern by hemoglobin electrophoresis.[1,2] The study groups were from same socioeconomic status with similar dietary history and were age matched with controls. Written informed consent was obtained from all the participants prior to the onset of the study. The pulmonary function test was done in all subjects using Medspiror-Medicare System, Ambala. The data of the subjects as regard to name, age, sex, height, weight, date of performing the test and atmospheric temperature were fed to the electronic Medspiror. All the parameters were measured as per the requirements of the American Thoracic Society guidelines for Spirometry.[9] Three consecutive readings were obtained for forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), FEV_1 as a percentage of FVC (FEV_1%), maximum mid-expiratory flow rate (MMEFR), peak expiratory flow rate (PEFR), and maximum voluntary ventilation (MVV). The average of the three readings was selected. Plasma malondialdehyde (MDA) was estimated by colorimetric method using 1 –methyl -2 Phenyl indole.[10-12] The whole blood superoxide dismutase (SOD) was measured by enzymatic method. Vitamin C (ascorbic acid) was measured by Colorimetric method.[12]

Statistical analysis of data

For data analysis all the values were expressed as mean ± standard deviation. Differences among the means were evaluated by one-way analysis of variance using GraphPad InStat (version 3, USA) software. *Post hoc* test was performed by Tukey-Kramer multiple comparison test. Correlation of data was done by Pearson's correlation using SPSS software (version 16 (SPSS Software Inc., Chicago, IL, USA). A difference was considered statistically significant if the probability of chance was less than 0.05 ($P < 0.05$).

RESULTS

Table 1 depicts the lung function parameters in SCD, SCT and controls. FVC, FEV_1, FEV_1%, MMEFR, PEFR and Vitamin C levels decreased significantly in both SCD and SCT but the decrease in FVC, FEV_1, FEV_1%, MMEFR and PEFR was more significant in SCD than SCT [Table 1]. Table 2 depicts the biochemical parameters in SCD, SCT and controls. MDA and SOD levels increased significantly in both SCD and SCT, but the increase was more significant in SCD than in SCT [Table 2]. Table 3 depicts the correlation of FEV_1% with biochemical parameters.

There was no significant correlation between FEV_1% with biochemical parameters in both SCD and SCT.

DISCUSSION

In the present study, FVC, FEV_1 and FEV_1% decreased significantly in both SCD and SCT but the decrease in FVC, FEV_1, and FEV_1% was more significant in SCD than SCT. Our findings were consistent with the reports of Sylvester *et al*.,[2] and Jaja *et al.*[5] which showed decreased FEV_1 in patients with HbSS with or without a history of ACS. MMEFR, PEFR and MVV decreased in SCD and SCT, though the extent of compromised function was more in SCD as compared with the traits and controls. The repeated chest infection in SCD and SCT was reported by our patients with an average incidence of three episodes of respiratory tract infections annually in SCD and SCT. This repeated infection over the years leading to change in geometry of pulmonary

Table 1: Comparison of pulmonary function profile in SCD, SCT and controls

Parameters	Control (*n*=45)	SCD (*n*=30)	SCT (*n*=45)	*P*
Age (years)	28.7±7.3	19.4±5.5***	24.5±7.5*.##	<0.0001
Height (cm)	169±4.2	162.7±4.5***	166.7±4.5###	<0.0001
Weight (kg)	65.3±4.8	58.1±5.5***	64.5±4.5###	<0.0001
FVC (L)	4.4±0.3	2.04±0.5***	2.7±0.58***.###	<0.0001
FEV_1 (L)	3.4±0.2	1.6±0.5***	2.20±0.36***.###	<0.0001
FEV_1%	81.2±1.6	77.9±3.1***	78.9±2.2***	<0.0001
MMEFR (L/s)	4.8±0.4	2.50±0.3***	3.2±0.3***.###	<0.0001
PEFR (L/s)	8.2±0.5	4.06±0.6***	4.8±0.3***.###	<0.0001
MVV (L/min)	130.2±16.7	72.8±15.3***	82.5±12.4***.#	<0.0001

Data expressed as mean±SD. SD: Standard deviation, SCD: Sickle cell disease, SCT: Sickle cell trait, FVC: Forced vital capacity, FEV_1: Forced expiratory volume in 1 s, MMEFR: Maximum mid expiratory flow rate, PEFR: Peak expiratory flow rate, MVV: Maximum voluntary ventilation. Statistical analysis was done by one-way ANOVA test followed by *post-hoc* Tukey test. The (*) depicts comparison with control: *P<0.05, **P<0.01, ***P<0.001. The (#) depicts comparison with SCD: #P<0.05, ##P<0.01, ###P<0.001

Table 2: Comparison of biochemical parameters in SCD, SCT and controls

Parameters	Control (*n*=45)	SCD (*n*=30)	SCT (*n*=45)	*P*
MDA (µmol/L)	1.82±0.22	4.16±0.3***	2.55±0.33***,###	<0.0001
SOD (U/ml)	198.16±22.4	320.44±18.64***	258.44±24.42***,###	<0.0001
Vitamin C (mg%)	0.94±0.1	0.43±0.42***	0.65±0.04***,##	<0.0001

Data expressed as mean±SD. SD: Standard deviation, SCD: Sickle cell disease, SCT: Sickle cell trait, MDA: malondialdehyde, SOD: superoxide dismutase. Statistical analysis was done by one-way ANOVA test followed by post-hoc Tukey test. The (*) depicts comparison with control: *P<0.05, **P<0.01, ***P<0.001. The (#) depicts comparison with SCD: #P<0.05, ##P<0.01, ###P<0.001

Table 3: Correlation of FEV_1% with biochemical parameters in control, SCD and SCT

	MDA		SOD		Vitamin C	
	r	*P*	*r*	*P*	*r*	*P*
Control	−0.13	0.3	0.1	0.4	−0.19	0.1
SCD	0.12	0.4	−0.03	0.8	0.12	0.5
SCT	0.46	0.1	0.10	0.4	0.11	0.4

FEV_1: Forced expiratory volume in 1 s, SCD: Sickle cell disease, SCT: Sickle cell trait, MDA: malondialdehyde, SOD: superoxide dismutase. Statistical analysis was done by Pearson's correlation. P<0.05 was considered to be statistically significant

parenchyma and physical properties of the elastic and collagen fibres and fibrotic lung parenchyma contributing to the compromised lung function as found in these patients.[6,7,13,14]

In the present study, MDA and SOD levels increased significantly in both homozygous, as well as heterozygous cases of SCA, but the increase was more significant in SCD than in SCT.[3,4,15,16] On the other hand, the levels of Vitamin C were significantly reduced in both homozygous as well as heterozygous cases of SCA but the decrease in Vitamin C was more significant in SCD than in SCT. Increased levels of MDA in both homozygous, as well as heterozygous cases of SCA, clearly indicate that SCA patients are exposed to conditions of augmented oxidative stress. Auto-oxidation is further compounded by the raised levels of SOD as observed in these patients. The raised SOD levels will result in the sickle cell erythrocytes being exposed to pathological amounts of hydrogen peroxide. The low levels of catalase reported in these patients will not be sufficient to protect the soluble cell components from oxidative injury.[3,8,16]

The pulmonary vascular bed is a highly vulnerable target organ for micro-vascular occlusion because its vasculature constricts with hypoxia in contrast to other vascular beds.[7] The ischemic reperfusion injury also leads to free radical generation. The pathophysiology behind lung damage is the hypoxia-driven adhesive-related occlusive events in the pulmonary microcirculation such as PHT and this may affect the diffusion and perfusion of gases and thereby reduces the lung functions in patients of SCD and SCT. This can be accompanied by decrease in the levels of the normal cytoprotective and adhesive mediator such as nitric oxide. High levels of superoxide ions can interact with nitric oxide with subsequent lowering of nitric oxide levels.[16]

The oxidative insult is further aggravated by the findings of significantly depleted levels of Vitamin C in this study. As discussed earlier, Vitamin C along with Vitamin E, plays an important anti-oxidant role in the body.[16] Sickle erythrocytes are continuously generating superoxide radicals and hydrogen peroxide. Hence, ascorbic acid and other anti-oxidants like Vitamin E are continuously utilized. Hence even with dietary intake similar to controls, sickle cell patients have low ascorbic acid levels due to increased utilization of these vitamins in detoxifying superoxide radicals and H_2O_2.[3,8,16] When the oxygen radical production exceeds the detoxifying capacity, these radicals would attack the RBC membrane leading to thiol oxidation and lipid peroxidation along with an accumulation of MDA. As discussed earlier, MDA accumulation can reduce red cell deformability and lead to the formation of irreversibly sickled red cells. These factors can contribute towards the painful micro-vascular occlusions seen in SCA patients. As SCA patients tend to be deficient in both Vitamin C and Vitamin E, their deficiencies may have additive effects, since Vitamin C and Vitamin E normally act synergistically to protect the cell membranes.

Furthermore, the reduction in the levels of these vitamins may be a factor for the increased susceptibility to infection reported in SCA. The increased red cell fragility and impaired red cell survival, degenerative changes in various organs including retina and increased susceptibility to infection seen in SCA may be associated with deficiencies in antioxidant vitamins.

Limitations of the study

We have not assessed the total lung capacity and diffusing capacity of carbon monoxide which could further help in understanding the pathophysiology associated with pulmonary ventilatory disorders. We have not correlated the pulmonary functions with hematological investigations and also, free radical levels with the hematological investigations to get a better insight on the role of severity of anaemia in deterioration of pulmonary functions and reduced oxidative defense in SCA and SCT. Though the subjects of all the three groups were young (15-45 years), they were not age and BMI matched.

CONCLUSION

The static and dynamic pulmonary functions such as FVC, FEV_1%, MMEFR, PEFR and MVV were reduced in SCD and SCT, and the decrease was more in SCD than in the traits. It was also evident that both homozygous, as well as heterozygous patients, are exposed to enhanced oxidative stress. It is also evident that the anti-oxidant system is unbalanced in these patients and is probably unable to effectively counteract the augmented oxidative stress to which the sickle cell erythrocytes are exposed. In future studies, we would like to see whether oral supplementation of anti-oxidant vitamins could help these patients to effectively counteract the oxidative damage.

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